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Problems in the Biochemical Specification of Neurons

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The idea that nerve fibers differ in their chemical constitution and that these chemical differences play an important role in determining the functional relations acquired by the growing fibers in development and regeneration has a long history extending back to the early writings of Langley (1), Cajal (2), and others at the turn of the century. The concept has received experimental substantiation during the past 25 years, particularly in the investigations of homologous response by Weiss (3) and in subsequent studies dealing with the growth and patterning of synaptic connections in various central nuclei (4). Not only has the existence of a widespread qualitative differentiation in the vertebrate nervous system been confirmed, but it has been shown further that this differentiation attains a much higher degree of refinement and specificity than was originally suspected. The evidence tells us, for example, that optic fibers arising from different points in the retinal field differ from one another in quality, that the same is true for cutaneous fibers innervating different spots in the skin, and for vestibular fibers supplying different points in the macula of the utriculus, and so on throughout many parts of the peripheral and central nervous system. In general, the chemical specificity seems to parallel closely the functional specificity and in some regions approaches in its refinement the level of the individual neuron.

It is clear from the data that the basic functional organization of the nervous system is closely linked to this qualitative specificity of the nerve cells. However, the exact manner in which the neuronal specificity gains expression in function remains uncertain. Some authors have proposed that the qualitative specificity determines the character of the impulses which the neurons conduct and the specific modes of excitation to which they will respond (5, 6). This possibility has yet to be proved or disproved. The most one can say at present is that all the evidence to date seems to be equally well explained by the more parsimonious assumption that the chemical differentials operate to determine the particular kind of synaptic associations, and to

a lesser extent, the kind of end organ attachments formed and maintained by the growing nerve fibers (4, 7). According to this interpretation the neuronal specificities assume paramount importance in the developmental organization of reflex pathways and also of higher level integrative mechanisms wherever the latter are patterned through inheritance rather than experience.

I. Nerve Specificity in Cutaneous Local Sign

The general concepts involved can be illustrated in terms of one of the more recently studied examples of neuronal specificity, namely, that which underlies cutaneous local sign. The term cutaneous local sign implies simply that something about the central effect of excitations arising from different points on the body surface enables one to locate the specific area of stimulation. In recent years it has been possible to learn something about its developmental organization from the results of various kinds of surgical disarrangement of the cutaneous nerve connections in rats, frogs, and salamanders (4, 7, 8, 20). The experimental procedures have included the cross union and central regeneration of cranial and spinal sensory roots, the deflection of peripheral nerves into foreign integument, the transplantation of limb buds, and the rotation of larval skin grafts through 180 degrees.

The findings all point to the same developmental picture, which is most simply outlined perhaps with reference to the recent observations of Miner (8) on cutaneous local sign in rotated skin grafts in the frog: If a strip of trunk skin extending from the midline of the dorsum to the midline of the

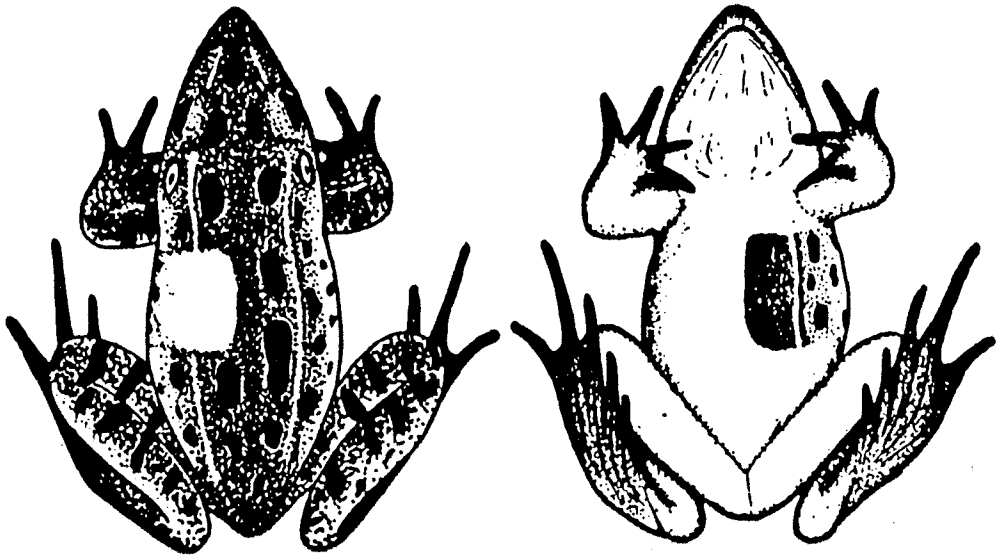

FIG. 1. Skin grafts cut free, rotated 180 degrees and reimplanted in tadpole stages. Localizing responses elicited from grafts are reversed, being directed with reference to intrinsic specificity of rotated skin [see Miner (8)].

belly is cut free, rotated 180 degrees, and reimplanted in early and midlarval tadpole stages, the graft undergoes a self-differentiation resulting in the growth of white belly skin on the dorsum and of black dorsal skin on the belly (see Fig. 1). After metamorphosis cutaneous localization in the graft is reversed. If the frog is stimulated on the dorsum, it rubs its belly with the foreleg. If stimulated on the belly, it wipes its back with the hindleg. These reversed responses remain uncorrected by reeducation.

The central reflex relations and local sign properties acquired by the cutaneous fibers are thus found to depend upon the kind of integument with which the fibers happen to connect in the periphery. In the present example the same cutaneous fibers form central reflex patterns for either the back-wiping reaction of the hindlimb or for the belly-wiping reaction of the forelimb, depending on whether the outgrowing peripheral endings happen to lodge in dorsal skin or in belly skin. Generally speaking, fibers that come to innervate a particular area of skin form central associations appropriate for that specific area. This is not regulated on any functional basis, for the development proceeds in the same prefixed manner, regardless of whether the effect be functionally beneficial, indifferent, or detrimental.

From these and related observations it has been inferred that the development of cutaneous local sign involves the following: (a) refined local specification of the integument of the entire body surface; (b) induction of a corresponding local specificity in the cutaneous fibers as a result of their contact with the skin, so that the individual fibers become stamped with the latitude and longitude, so to speak, of their termination in the cutaneous field; (c) qualitative differentiation of the central, second-order neurons with which the cutaneous fibers make synapsis; and (d) selective establishment of central synaptic associations governed by specific chemical affinities between the sensory and central neurons.

II. Problems

This series of events raises a host of problems at the molecular level, most of which can be formulated at present only in speculative terms. With regard, first, to the differentiation of the integument, the results indicate that each spot in the skin must acquire a unique chemical make-up that distinguishes it from any other cutaneous locus except possibly for the corresponding spot on the opposite side of the body. One does not picture a punctate mosaic of different qualities, but rather a smooth, axial gradient of differentiation, the slope of which is steepest in areas of most refined sensitivity. A single anteroposterior gradient or a single dorsoventral gradient by itself would not be sufficient. However, a dorsoventral gradient superimposed transversely upon a qualitatively distinct anteroposterior gradient on each side

of the body would be adequate to endow each point in the two-dimensional cutaneous field with a unique combination of properties. This would also satisfy the requirement that points far away from each other in the integument be more dissimilar than points near together and further that the local sign specificity of the skin reflect in an orderly maplike fashion the spatial interrelations of all points with one another.

The foregoing would require at the most only two qualitatively distinct chemical factors, each capable of a graded intensity varying smoothly from point to point across the skin. This is the simplest explanation we can postulate, and it fits the concept of fields and axial gradients as applied generally to embryonic differentiation (9). The actual situation is probably more complex. Certainly there is evident in the integument a differentiation along the radial or surface-depth dimension, and, of course, the simple axes of the primitive fishlike body surface are grossly modified in the more complicated contours of the four-limbed vertebrates, necessitating mediolateral, proximodistal, and possibly other gradients of differentiation in some regions. However, the basic idea still applies.

The process by which the local sign specificity of the integument becomes stamped upon the cutaneous fibers presents a second major problem. We have assumed this to be another case of developmental induction, of which many types and examples are well known in embryology. It can be inferred that the inductive process must take place only at the peripheral tips of the fibers, for the fibers follow devious and erratic routes through the subcutaneous tissues and cutaneous plexuses to their sites of termination. Were they subject to inductive influences from outside contacts throughout their course, the precision of cutaneous sensibility would be lost. For this and other reasons an intimate and stable contact between the fiber tips and the integument would seem to be a necessary condition. The region of functional contact of a nerve cell with its end organ or with other neurons probably involves more than mere contact, however close, of the two cell surfaces. We picture a partial membrane breakdown with molecular rearrangements to favor passage of cytoplasmic influences across the interface. Unlike most of the neuronal membrane, these special points of functional contact may offer no greater obstacle to interaction than does the interface between nucleus and cytoplasm. The exact mechanism by which the local cutaneous specificity or its complement becomes transmitted to, molded upon, or synthesized in the nerve fibers remains a matter for speculation.

Another problem relates to the mechanism by which the local specificity is spread from the peripheral fiber tips into the central arborizations within the cord and brain. Axoplasmic elements, after being stamped with the local specificity at the peripheral fiber tips, might travel by diffusion and other forces into the central ramifications. Or the peripheral influence might first

act upon the nucleus causing synthesis in this locality of specific molecular complexes which then are transported throughout all processes of the neuron. Or some kind of chain reaction might start at the periphery to spread along the fiber surface or its interior. Any combination of these and other mechanisms could be involved, for there is little in the present evidence to restrict speculation. Transport along the axis cylinder of the poliomyelitis virus (10) and of tetanus toxin at the rate of 3.35 mm/hr or faster (11) may be relevant. In any case, it would seem essential that the specificity be extended into the very tips of the hundreds of central collaterals of each cutaneous fiber with no loss or adulteration of the precise local specificity imposed at the periphery.

Perhaps the central problem from the neurochemical standpoint concerns the chemical character of the specificity itself. It should be remembered in this connection that any single neuron is commonly tagged with more than one kind of specificity. In the cutaneous system the fibers are differentiated as pain, touch, warm, or cold fibers, in addition to carrying the local sign specificity, which in itself involves differentiation with reference to at least two axes. One would expect to find the chemical factors underlying the modal and the axial specificities to be qualitatively distinct involving widely differing molecular structure. On the other hand, determination of the exact locus along a given axis in the skin would seem to require differing doses or intensities of the same chemical factor. We are inclined at present to picture large molecules or molecular complexes which as units undergo exact replication and the chemical properties of which can be adjusted through a wide range of minute gradations. Molecule density or concentration as a basis of the gradients seems to be eliminated by the neces-

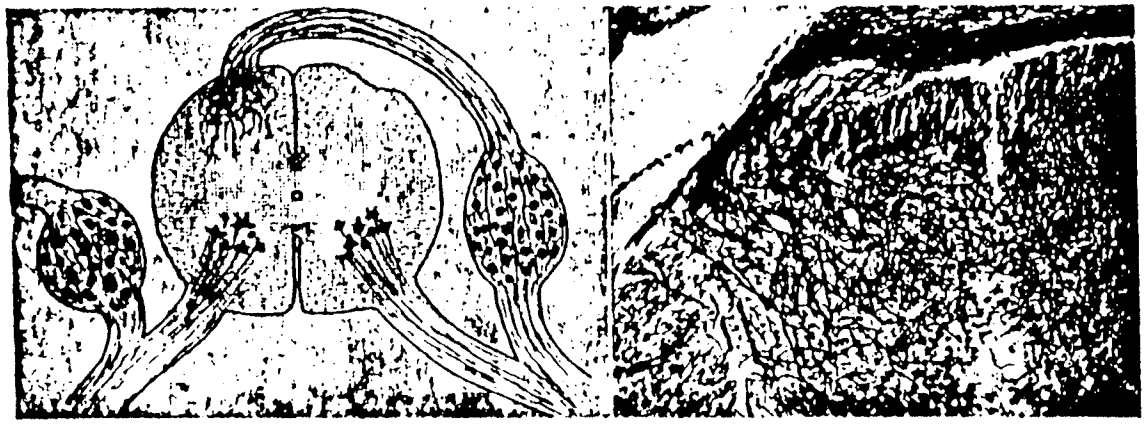

FIG. 2. Diagram and photomicrograph of dorsal root fibers cut and cross-connected to contralateral limb segments of cord in frog tadpole. Regenerated fibers established orderly reflex relations despite their chaotic intertangling and the maladaptive functional effect [from Sperry (4)].

sity for extensive spread into numerous and widely separated central collaterals without alteration or loss of specificity.

Further neurochemical problems are encountered in the selective formation of synaptic linkages in the centers. It has seemed a reasonable assumption that the central ramifications of the sensory root fibers tolerate synaptic relations only with a certain small fraction of the various kinds of central cells with which they make contact (see Fig. 2). To a degree this kind of selectivity is generally evident, in that the sensory fibers do not form synaptic endings on the glial cells, the blood capillaries, or on other axons which they encounter. Contact with a very special kind of neuron surface seems necessary to cause the growing fiber tips to adhere firmly, to cease further elongation and to form a lasting, functional synapse. The requisite specification of the central neurons which is assumed here would seem to involve no phenomena of a radically different category from those underlying differentiation in the peripheral ganglia and integument.

The kind of chemoaffinity required for the selective linkage of sensory and central neurons is not unlike that observed among other biological phenomena, a number of examples of which are well known in the laboratory. We know that if a single species of motile sperm is introduced into a dish of sea water containing a wide variety of unfertilized eggs, the sperm will adhere to and fertilize only those egg cells of its own kind. If eggs of several closely related species happen to be included, cross fertilization may occur in a consistent, predictable spectrum (12). Similarly, if a series of different types of bacteria are exposed to a single bacteriophage, the virus units adhere to (13) and multiply selectively only on the particular bacteria for which the phage is specific. Dissociated cells of a sponge recombine selectively with their own kind (14). Other examples can be found in the fields of immunochemistry and of plant physiology. The selective chemoaffinities involved in synaptic formation in the nervous system are special only in their extreme refinement. They go beyond the order of species, tissue, and even of organ specificity, almost, in some instances, to that of the individual cell. Mechanisms postulated to account for selective adhesion in other biological phenomena (13, 15, 16, 17, 18) may apply to the patterning of neuronal linkages. In particular, there are suggestions (12, 15, 16) that the same assortment of physicochemical forces involved in the antigen-antibody reaction may underlie many other biochemical affinities, including those of neuronal synapsis.

Each cutaneous fiber presumably forms synaptic terminals on several different classes of central neurons, with the particular spectrum of central terminations varying in accordance with the local sign and modal specificity of the fiber. Such spectral termination certainly is more common in the central nervous system than is termination restricted to a single cell type.

One assumes also that there is extensive overlap in the central terminations of neighboring cutaneous fibers upon any given class of internuncials much like the overlap in the peripheral terminations (19, and see Fig. 3). It is conceivable that the same fiber may form synaptic junctions that are excitatory on one class of neurons and inhibitory on neurons functionally antagonistic depending on how the endfeet engage the soma membrane. In the earlier stages of development the local specificity impressed on the cutaneous fibers may be shifted by changing the end-organ connections. Later the specificity either becomes irreversibly fixed or else its change is not capable of causing readjustment in the central synapses. The period and degree of embryonic plasticity seem to become increasingly restricted as one ascends the vertebrate scale (3, 7, 20).

The foregoing discussion is confined to cutaneous local sign for purposes of brevity and concrete illustration. Similar problems exist with reference to the developmental patterning of all the sensory and motor systems and also of the integrative circuits within the centers. At this date we can only point

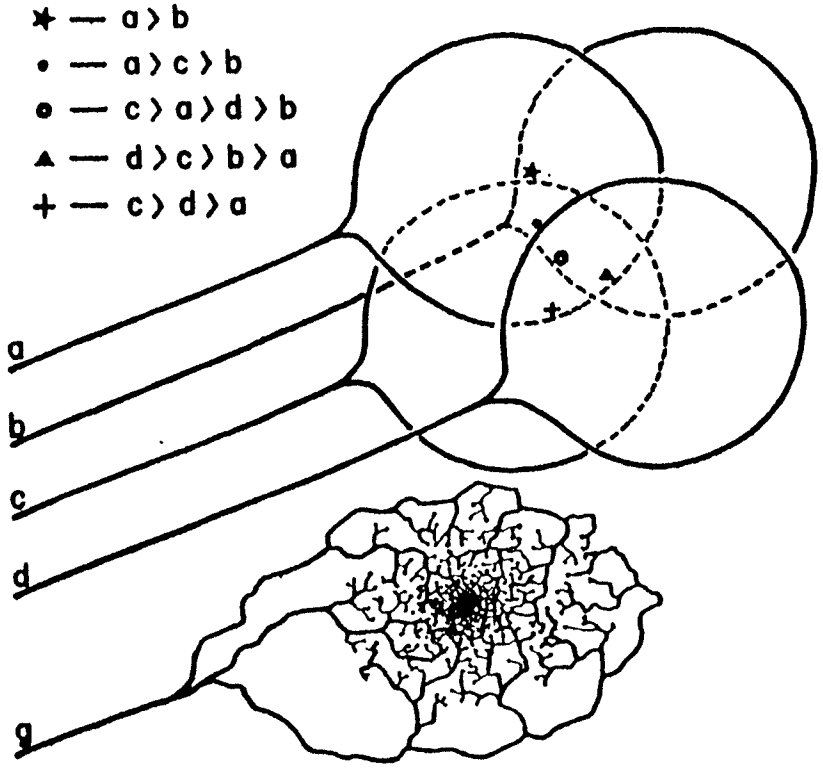

FIG. 3. Schematic diagram of peripheral overlap in neighboring cutaneous fibers, to illustrate how stimulation at points close together might produce different patterns of central discharge distinctive for locus of stimulation and indicative of spatial interrelationships. In addition to assumptions made in text, it is presumed that distribution of each fiber is focalized (note fiber g) such that frequency of discharge evoked by a given stimulus falls off from center toward periphery (21).

up some of the many neurochemical problems; in most cases the answers lie far beyond us.

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DISCUSSION

S. S. KETY: Dr. Sperry's work is always provocative, and this is no exception. I would like to focus attention on the transplanted limbs of the frog, and ask if you have any information upon the manner in which the motor fibers grow out from the central nervous system and into the transplanted muscle, and the sensory fibers travel from the sensory endings into the nervous system. Which one reaches its destination first, and what do you think are the sequence of events in that consideration?

R. W. SPERRY: I should apologize there for eliminating one sentence in which I planned to say that all those reactions are made by the normal ipsilateral limb but the transplanted limb shows no active function of its own. So it simply acts as a sensory field for these fibers to terminate in. Responses are all on the normal limb.

H. P. WHITING: I have been very interested in the extension of Dr. Weiss's theory which Dr. Sperry has put forward, in regard to the modification of the synapse centrally as a result of the positions in which the axon or any peripheral fiber finds itself. Does he suppose if the limb is grafted into a new position, say, three segments above or three segments below, that the dendrites of the central connections now to be

made are going to extend three segments up or down the cord corresponding with the position of the new peripheral terminations? If he considers that the chemical field in the periphery which he envisages is spread over the whole skin—as he put it, with a longitude and latitude—then he must suppose that essentially there must be a traveling of the dendrites up or down the cord for a distance which will bring the dendrites into the position of the new piece of skin or muscle to which the axon is attached.

R. W. SPERRY: In the case of the sensory fibers under discussion, the central processes in the cord typically divide into large ascending and descending branches that may travel the entire length of the cord, sending down small collaterals at many segmental levels. Under these conditions there is no problem of getting the fibers into contact centrally with the different limb and other segments. On the motor side, one has to know exactly which three segments are involved. If they are limb segments, very possibly the dendrites of the motor cells do extend sufficiently far, and in any case it is not necessarily a matter of dendritic outgrowth, for terminals of the second-order cells may spread rather diffusely through the whole center.

P. GLEES: Was the transplanted and reversed skin innervated before transplantation? If so, terminating skin fibers would degenerate and might produce, as a degenerative product, a specific attracting substance which attracted only that type of fiber which previously innervated it.

R. W. SPERRY: There is undoubtedly some innervation at the time that the transplant is made and probably some new fibers are added later; very possibly the new innervation follows degenerated pathways to some extent. This does not much simplify, however, the problem of attaining precise functional adjustment between the central and peripheral connections.

V. HAMBURGER: I should like to point out that the specificities which Dr. Sperry just described far surpass all the specific responses to peripheral factors, which I discussed yesterday. The question I should like to ask is what are the origins of these specificities? According to your statement, the primary specification is in the skin or in the effector region. On the other hand we have to assume that regional specificities self-differentiate during the development of the central nervous system. For instance, it is very significant that in all limb transplantations in amphibians or birds, functional activity can be obtained only if the transplanted limb is connected with a limb region of the spinal cord. We apparently deal with a matching of specificities engrained in nonnervous structures, on the one hand, and specificities in the nervous system. Is it your idea that the former become superimposed on the latter?

R. W. SPERRY: In the case of the cutaneous fibers and their ganglion cells, very probably those fibers undergo an independent preliminary differentiation into pain, tactile, cold, and warm fibers—but the local sign specificity would have to be imposed from the periphery. In general the final specificity must be a product of two processes: self differentiation and the induction of specificity from related tissues. Our interpretation implies that the central neurons on which the cutaneous root fibers terminate must undergo an equally refined differentiation with reference to their afferent and motor relationships. The centers in general must undergo a more complex pattern of differentiation than does the peripheral nervous system. There is some direct evidence for this in the regeneration of tectospinal and tectobulbar tracts of the visual system.

R. W. GERARD: I have had the pleasure of watching this work develop over almost all its entirety, through contact with Drs. Weiss and Sperry. The first time I met

Paul Weiss, in Coghill's laboratory, we got into violent argument about the resonance theory and hardly talked with our host. At that time the work of Sherrington and Adrian was just proving that motor and sensory frequencies vary with intensity, so that a "resonance" of input and output of matching discharge frequencies was untenable. The chemical interpretation in some ways is even more dramatic and revolutionary. I am not happy with it, but can't see anything to do except go along until somebody thinks of some alternative interpretation. You may be in a bit of trouble with so many specifications operating potentially on the same neurons or neural patterns. If specifications were controlled only on the sensory side or only on the motor side or only centrally, you'd have a fairly airtight situation (indeed, you may have anyway), but with multiple determination I think conflict could arise. Have you tried, or if you haven't would it seem worth trying, to reverse the skin and at the same time reverse one of the limbs or its muscles so as to effectively reverse the motor output? What kind of final specification would result where cord neurons were subjected to two abnormal patterns affecting the same reflex system?

R. W. SPERRY: One could only predict that the different types of cells and their interconnections would all sort out properly. One would not expect translocations of the limbs or muscles to affect the chemical properties of the cutaneous field.

S. S. KETY: I'm afraid that Dr. Gerard, by a curious nonchemical type of telepathy, has answered the question I was about to raise, but since I didn't understand his rather laconic reference to the resonance theory I'm foolish enough to go ahead and suggest it anyhow. It is not necessary to presuppose a chemical specificity but a frequency specificity, so that this nerve fiber, as it runs along, may not be searching for a specific chemical mate, but for a specific tone. What has been suggested was that the entire organization goes up and down the spinal cord in the fullblown reflex, only the appropriate one affecting its appropriate mate, but could not this developmental process take place just as Dr. Sperry suggested but with a frequency-searching instead of a chemical one? One other question—have neuroanatomical techniques reached the point where it is possible to demonstrate these connections?

R. W. SPERRY: In the latter case, no, unless perhaps Dr. Whiting can see the synaptic relations in very simple reflex arcs in the early stages of development. In some ways the neuropil of the amphibians and fishes is more complicated and difficult to analyze than that of the mammals. The majority of the synaptic connections are made in the white matter and are most difficult to untangle. If I understand your first question, it leaves the problem that frequencies vary along a single dimension, and one needs greater differentiation of cell types than this could provide. As you know, the actual frequencies of motor neuron discharge show a range of maybe 5 or 6 up to around 60, but those for all the muscles fall roughly in the same range. The same is true on the sensory side, though the frequencies go much higher. Even if one could distinguish one fiber from another on a frequency basis, I doubt if it would be possible to locate them correctly with reference to a two-dimensional plane, with all their spatial interrelationships accurately reflected in the central connections on such a basis.

J. E. HARRIS: I am a little out of my depth with some of the more elaborate examples that have been quoted, but I would like to challenge the interpretation of Dr. Sperry's first experiment, which was a beautifully elegant one. It seems to be assumed that in transposing this piece of skin through 180° you have done nothing but alter cutaneous sensory areas of the animal. But that skin contains, surely, a considerable number of effectors, namely, chromatophores, and those chromatophores retain their

graded "dorsoventral" pattern in the new reversed position. There exists, consequently, a possibility of a new reflex pattern which may match up with the normal dorsoventral relationships of the animal. This would produce precisely the results you describe without the need for an elaborate chemical specification.

R. W. SPERRY: I am not sure I understand. Tell me where the interpretation goes wrong. The ventral fibers grow into the dorsal skin, whereupon, instead of forming ventral patterns of central reflex connections as usual, they form dorsal patterns.

J. E. HARRIS: Yes, but the reason, I think, might be rather different from the reason you suggest. It is because these mixed ventral fibers now meet chromatophore-containing skin, and hence can establish reflex patterns with those chromatophores just as if it were a normal dorsal piece of skin.

R. W. SPERRY: If I follow correctly, your interpretation would apply to such local autonomic responses as might be confined entirely within the rotated skin following stimulation that also was confined to the graft. However, it is difficult to see how the observed skeletal reactions—of the limbs, for example—could be explained in these terms.

J. D. BOYD: Like Dr. Gerard, I feel unhappy about the explanation of these beautiful experiments, but I don't see the snag. Is it possible that part of the explanation might be found in atypical regeneration of the nerve supply to the reversed skin graft? To what extent, one would like to know, was there a collateral invasion of the reversed skin by fibers from the surrounding skin?

R. W. SPERRY: Approximately two to four dermatomal segments are included in the grafts. There is some invasion of the graft by fibers from the neighboring intact skin. In line with this, the reactions from the graft are not pure; some normal reactions occur, varying in number in different animals. By making a fresh cut around the graft a few days before testing, most of the impure (normal) responses can be eliminated. Others may arise from uncontrolled subcutaneous stimulation.

The data are quite compatible with an interpretation expressed in terms of some kind of specific nerve energies. In the present state of neurophysiology and neuroanatomy, however, one feels obliged to try to account for the phenomena, if at all possible, in terms of connections. If we are eventually forced to do otherwise, fine; but this would mean you see, a rather extensive revamping of the classic concepts of neural integration.